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Dietary and Other Methyl-Group Availability Factors and Pancreatic Cancer Risk in a Cohort of Male Smokers

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The authors examined prospectively whether dietary folate and other factors known to influence methyl-group availability were associated with the development of exocrine pancreatic cancer within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort. Of the 27,101 healthy male smokers aged 50–69 years who completed a self-administered dietary questionnaire at baseline, 157 developed pancreatic cancer during up to 13 years of follow-up from 1985 to 1997. Cox proportional hazards models were used to estimate the hazards ratios and 95% confidence intervals. The adjusted hazards ratio comparing the highest with the lowest quintile of dietary folate intake was 0.52 (95% confidence interval: 0.31, 0.87; *p*-trend = 0.05). Dietary methionine, alcohol intake, and smoking history did not modify this relation. No significant associations were observed between dietary methionine, vitamins B_6 and B_{12}, or alcohol intake and pancreatic cancer risk. Consistent with prior studies, this study shows that cigarette smoking was associated with an increased risk (highest compared with lowest quintile, cigarettes per day: hazards ratio = 1.82; 95% confidence interval: 1.10, 3.03; *p*-trend = 0.05). These results support the hypothesis that dietary folate intake is inversely associated with the risk of pancreatic cancer and confirm the risk associated with greater cigarette smoking. *Am J Epidemiol* 2001;153:680–7.

alcohol drinking; folic acid; methionine; pancreatic neoplasms; pyridoxine; smoking; vitamin B 12

Cancer of the exocrine pancreas accounts for 2 percent of the malignancies worldwide and is among the most rapidly fatal cancers (1), ranking fifth for cancer mortality in the United States (2). Because there is no effective screening for pancreatic cancer, most cases are diagnosed at advanced stages and have a 5-year survival of <5 percent (2). With the exception of cigarette smoking and age, few consistent risk factors have been identified (3). A recent report by the World Cancer Research Fund and the American Institute for Cancer Research estimated that 30–50 percent of pancreatic cancer may be attributed to dietary factors (1), although the specific dietary components remain unclear because of limited and inconsistent study findings. Consumption of a diet high in fruits and vegetables was among the most probable dietary factors associated with protection from pancreatic cancer (1). This protective association with fruits and vegetables, the major dietary folate sources, suggests a potential role for dietary factors that influence methylation in the development of pancreatic cancer.

Folate, vitamin B_{12}, vitamin B_6, and methionine are dietary methyl-group-related factors involved in the deoxyribonucleic acid (DNA) methylation and DNA synthesis pathways. Diminished methyl-group status due to reduced availability of *S*-adenosylmethionine for DNA methylation may increase the susceptibility of genes to mutations (4) and affect gene expression (5, 6). Another suggested mechanism posited for inadequacy of these nutrients in carcinogenesis is less available methylenetetrahydrofolate for methylation of deoxyuracil monophosphate to deoxythymine monophosphate, resulting in misincorporation of uracil for thymine in DNA (7) and greater potential for chromosome strand breaks and/or DNA excision repair (8). Diets having lower methyl-group availability (high alcohol, low folate, and/or low methionine) have been associated with colorectal and breast cancer (9–12) and may also be associated with pancreatic cancer. Heavy consumers of alcohol tend to have low folate status and their folate intake is reduced (13). In addition, cigarette smoke may influence methyl-group availability by reducing folate and vitamin B_6 status and interfering with vitamin B_{12} metabolism (14–19).

In a nested case-control study, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, we previously reported a significant halving of pancreatic cancer risk among smokers associated with a greater fasting serum folate (odds ratio = 0.45; 95 percent confidence interval:

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Abbreviations: ICD-9, *International Classification of Diseases*, Ninth Revision; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

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0.24, 0.82; p-trend = 0.009) and pyridoxal-5′-phosphate (odds ratio = 0.48; 95 percent confidence interval: 0.26, 0.88; p-trend = 0.02) concentration compared with those with the lowest concentration, as well as an independent twofold increased risk associated with greater cigarette smoking (20). The purpose of the present study was to examine whether dietary folate and other factors known to influence methyl-group availability are associated with the development of exocrine pancreatic cancer in a large prospective cohort study.

MATERIALS AND METHODS

Study cohort

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study was a double-blinded, placebo-controlled, 2 × 2 factorial design, primary prevention trial that tested whether alpha-tocopherol or beta-carotene reduced the incidence of lung cancer in male smokers (21). Between 1985 and 1988, 29,133 eligible men aged 50–69 years who smoked at least five cigarettes per day were randomized to receive active supplements (alpha-tocopherol, 50 mg/day; beta-carotene, 20 mg/day; or both) or placebo in southwestern Finland. Men were excluded from the study if they had a history of malignancy other than nonmelanoma cancer of the skin or carcinoma in situ, severe angina on exertion, chronic renal insufficiency, liver cirrhosis, or chronic alcoholism; were receiving anticoagulant therapy or had other medical problems that might limit long-term participation; or were currently taking supplements containing vitamin E (>20 mg/day), vitamin A (>20,000 IU/day), or beta-carotene (>6 mg/day). Compliance with the intervention for the trial was ~95 percent, as determined by residual capsule counts and confirmed by serum measures of alpha-tocopherol and beta-carotene (21). The trial ended April 30, 1993, and follow-up continued after randomization until death or through November 1997, representing follow-up data for up to 13 years (median, 10.2 years) for the present study. The study was approved by the institutional review boards of both the National Public Health Institute in Finland and the US National Cancer Institute, and all study participants provided written informed consent prior to the study's initiation. Details of the study rationale, design, and methods have been described previously (21). As methyl-group-related nutrients are metabolized in a similar manner in men and women, the results from this study should be applicable to women.

Baseline characteristics, smoking, and dietary factors

The study participants completed questionnaires on general background characteristics including medical, smoking, and dietary history at their baseline visit. Diet was assessed with a self-administered dietary history questionnaire, which determined the frequency of consumption and usual portion size of 276 food items during the past year, using a color picture booklet as a guide for food items and portion size (21). The questionnaire was linked to a food composition database of the National Public Health Institute in Finland. The dietary history questionnaire was developed for the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, and its correlation coefficients for validity and reliability for nutrients and foods ranged from 0.40 to 0.80 and 0.56 to 0.88, respectively (11, 22).

Case ascertainment

Pancreatic cancer cases were ascertained from the Finnish Cancer Registry, which provides almost 100 percent case ascertainment in Finland (23, 24). All relevant medical records for reported incident pancreatic cancer cases were reviewed independently by two study oncologists (21). Only cases confirmed by the study physicians as incident primary malignant neoplasms of the exocrine pancreas (*International Classification of Diseases*, Ninth Revision (ICD-9), code 157) (25), from randomization through November 1997, were used for the present analysis. Approximately 79 percent of these cases had a histopathologic diagnosis assigned centrally by the study pathologists after examining pathology and cytology specimens (21). Islet cell carcinomas (ICD-9 code 157.4) (25) were excluded, as their etiology may be different from that of the exocrine tumors. There were 168 confirmed exocrine pancreatic cancer cases, of whom 157 had completed the dietary questionnaires at baseline.

Statistical analysis

The follow-up time for each participant was calculated from the date of randomization until diagnosis of pancreas cancer, death, or November 1997, totaling 259,918 person-years of observation. Only those with complete dietary and smoking history (n = 27,101) were included in the analyses. Variables were analyzed as both continuous and categorical variables with the latter based on the distribution in the entire cohort. Trends in categorical variables were tested as a calculated score variable based on the median values of each category. Supplemental folic acid, vitamin B_{12}, and vitamin B_6 were categorized as dichotomous yes/no variables based on reported use at study entry. Categories for the smoking inhalation variable were defined as never/seldom, often, and always. Pack-years were estimated by multiplying the years smoked by the average number of packs smoked per day at baseline. Smoking cessation was reported during the trial through April 1993 and was defined as having reported quitting for at least three consecutive follow-up visits during the trial (i.e., 1 year) for this analysis.

Spearman's correlations were performed to assess correspondence between the study variables. As the primary nutrients of interest were highly correlated to energy (folate, r = 0.82; vitamin B_6, r = 0.77; vitamin B_{12}, r = 0.51; and methionine, r = 0.82), all dietary nutrients were energy adjusted using the residual method described by Willett and Stampfer (26), using log-transformation to preserve the linear model assumption. Alcohol intake was not energy adjusted because it was not strongly correlated with energy (r = 0.11). Analyses were performed separately for dietary and supplemental nutrients. As many of the variables had skewed distributions, the characteristics of the cases and

noncases, as well as the characteristics of vitamin users versus non-vitamin users, were compared using nonparametric tests (Wilcoxon's rank sum test and chi square). Hazards ratios and 95 percent confidence intervals were determined using proportional hazards models. Multivariable models were developed separately for each nutrient and smoking variable by individually adding covariates to the model in a stepwise fashion and were all controlled for the study interventions, alpha-tocopherol and beta-carotene. Variables were considered confounders if they were associated with both the disease and the risk factor and if they changed the risk estimate from the crude ≥10 percent. Variables examined as potential confounders in the analyses included all those listed in table 1, as well as dietary fiber, height, weight, and education. The dietary variables were introduced into the models as score trend variables of their energy-adjusted residual quintile and other variables as continuous or categorical variables. Effect modification was tested through cross-product terms in the multivariable models and estimates of stratified hazards ratio. All statistical analyses were performed using Statistical Analysis System (SAS) software, and statistical tests were two-tailed.

RESULTS

Baseline characteristics of the cohort by quintile of energy-adjusted folate intake are shown in table 1. Men with greater dietary folate intake (relative to kilocalories) tended to have a higher body mass index; to have higher intakes of protein, methionine, vitamins B_{12}, B_6, C, and E, carotenoids, and selenium; to drink less alcohol; to have a shorter smoking duration and a less cumulative smoking dose; to inhale their cigarettes less often; and more often to have a history of diabetes. At entry into the cohort, cases

TABLE 1. Baseline characteristics (medians and proportions) by energy-adjusted dietary folate intake, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1997*

Characteristics	Quintile of energy-adjusted folate intake (μg/day)				
	<280	280–310	311–338	339–373	>373
Age (years)	57	57	56	56	56
Body mass index (kg/m^2)	25.7	25.8	25.9	26.1	26.4
Dietary intake†					
Energy (kcal/day)	2,569	2,733	2,758	2,768	2,724
Alcohol (g/day)	16.0	12.5	11.1	9.8	7.9
Protein (g/day)	92	98	101	103	105
Methionine (mg/day)	1,853	1,947	2,000	2,043	2,081
Folate (μg/day)	257	295	324	354	400
Vitamin B_{12} (μg/day)	8.82	9.77	10.24	10.75	11.50
Vitamin B_6 (mg/day)	2.08	2.30	2.42	2.55	2.76
Vitamin C (mg/day)	63	78	88	100	121
Vitamin E (mg/day)	8.33	9.40	10.18	11.02	12.45
Carotenoids (mg/day)	2.67	3.33	3.92	4.49	5.67
Selenium (μg/day)	82	85	87	88	89
Supplement intake					
Folic acid (%)	5.1	5.6	5.8	6.1	6.9
Vitamin B_{12} (%)	6.3	6.8	7.4	7.4	8.9
Vitamin B_6 (%)	11.6	12.6	12.4	12.7	15.3
Smoking history					
Total cigarettes smoked daily	20	20	20	20	20
Years smoked (no.)	38	37	36	35	35
Pack-years (no.)	40	38	35	33	32
Age started smoking (year of age)	18	19	19	19	19
Smoking inhalation					
Never/seldom (%)	6.7	8.5	8.8	9.5	11.3
Often (%)	30.3	36.7	38.6	41.7	43.1
Always (%)	63.0	54.8	52.6	48.7	45.5
Disease history					
Diabetes (%)	2.7	3.3	4.0	4.6	6.5
Pancreatitis (%)	1.8	1.4	1.3	1.6	1.1
Gallbladder disease (%)	5.2	5.0	5.8	5.5	6.5

* Elementary school was the median level of educational attainment for all folate quintiles.
† Dietary nutrients adjusted for energy.

compared with noncases were older (median age, 58 vs. 57 years; $p = 0.0001$); had greater smoking years (median, 40 vs. 36 years; $p = 0.002$) and pack-years (median, 39 vs. 35 years; $p = 0.03$); and had lower energy-adjusted dietary folate (median, 315.5 vs. 323.8 g/day; $p = 0.05$) and energy (median, 2,612 vs. 2,721 kcal/day; $p = 0.04$) intake. Cases did not differ significantly from noncases with respect to body mass index; energy-adjusted vitamin C, vitamin B_6, vitamin B_{12}, protein, and methionine intake; alcohol consumption; and other dietary or smoking variables. The median age at diagnosis of pancreatic cancer was 64 years (range, 50–78 years).

In the multivariable models, the relation of folate to pancreatic cancer risk was not confounded by age; body mass index, height, or weight; intervention; education; alcohol, supplement, or energy-adjusted protein; methionine, intake of vitamins B_6, B_{12}, C, and E, selenium, or carotenoid; smoking habits; or disease history. Age was a confounder of dietary vitamin B_6, alcohol intake, number of cigarettes smoked daily, years smoked, and smoking inhalation; and dietary folate was a confounder of dietary vitamins B_6 and B_{12} and methionine but not of alcohol. No other variables examined were confounders in the models. Therefore, table 2 displays the age-, intervention-, and folate-adjusted models, and table 3 displays the age- and intervention group-adjusted models.

Baseline energy-adjusted dietary folate was significantly inversely associated with pancreatic cancer risk with those in the highest quintile having approximately half the risk compared with those in the lowest quintile and with a significant trend (table 2). Among folate-rich foods, nonsignificant modest inverse associations were observed for energy-, intervention group-, age-, and smoke year-adjusted cruciferous vegetables (highest compared with lowest quin-

TABLE 2. Hazards ratios of pancreatic cancer according to categories of energy-adjusted dietary folate, vitamin B_6, vitamin B_{12}, and methionine from study randomization, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1997

Nutrient and quintile of energy-adjusted intake	Cases (no.)	Person-years (no.)	Energy-adjusted hazards ratio	95% confidence interval	Multivariate hazards ratio	95% confidence interval
Folate (μg/day)						
1 (≤280)	43	50,587	1.00	Reference	1.00*	Reference
2 (281–310)	29	51,815	0.66	0.41, 1.05	0.67	0.42, 1.08
3 (311–338)	25	52,293	0.56	0.34, 0.92	0.59	0.36, 0.96
4 (339–373)	38	52,629	0.85	0.55, 1.31	0.89	0.57, 1.37
5 (>373)	22	52,594	0.49	0.29, 0.82	0.52	0.31, 0.87
				p-trend = 0.03		*p*-trend = 0.05
Vitamin B_6 (mg/day)						
1 (≤2.09)	34	50,772	1.00	Reference	1.00†	Reference
2 (2.10–2.33)	28	51,747	0.81	0.49, 1.33	0.92	0.55, 1.53
3 (2.34–2.54)	35	52,124	1.00	0.62, 1.60	1.25	0.76, 2.05
4 (2.55–2.81)	29	52,586	0.82	0.50, 1.34	1.11	0.65, 1.90
5 (>2.81)	31	52,689	0.87	0.54, 1.42	1.32	0.75, 2.30
				p-trend = 0.61		*p*-trend = 0.28
Vitamin B_{12} (μg/day)						
1 (≤7.57)	35	52,131	1.00	Reference	1.00†	Reference
2 (7.58–9.26)	25	51,865	0.72	0.43, 1.20	0.73	0.44, 1.22
3 (9.27–11.08)	40	51,678	1.15	0.73, 1.82	1.21	0.77, 1.90
4 (11.09–13.68)	30	51,899	0.86	0.53, 1.40	0.93	0.57, 1.53
5 (>13.68)	27	52,345	0.77	0.47, 1.27	0.88	0.53, 1.48
				p-trend = 0.47		*p*-trend = 0.91
Methionine (mg/day)						
1 (≤1,720)	32	51,745	1.00	Reference	1.00†	Reference
2 (1,721–1,904)	32	51,930	1.00	0.61, 1.63	1.03	0.63, 1.69
3 (1,905–2,068)	39	52,383	1.20	0.75, 1.92	1.30	0.81, 2.09
4 (2,069–2,268)	26	52,150	0.81	0.48, 1.35	0.88	0.52, 1.49
5 (>2,268)	28	51,710	0.88	0.53, 1.45	1.00	0.60, 1.68
				p-trend = 0.47		*p*-trend = 0.88

* Adjusted for age (continuous) and intervention (alpha-tocopherol and beta-carotene supplement).

† Adjusted for age (continuous), intervention (alpha-tocopherol and beta-carotene supplement), and energy-adjusted folate (quintile trend variable).

TABLE 3. Hazards ratios of pancreatic cancer according to alcohol intake, supplement use, and smoking habits from study randomization, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1997

Risk factor	Cases (no.)	Person-years (no.)	Crude hazards ratio	95% confidence interval	Multivariate hazards ratio*	95% confidence interval
Alcohol intake						
None	14	28,201	1.00	Reference	1.00	Reference
Any	143	231,717	1.24	0.71, 2.14	1.35	0.78, 2.35
Alcohol intake (g)						
None	14	28,201	1.00	Reference	1.00	Reference
>0–≤5.3	39	56,847	1.37	0.75, 2.53	1.39	0.75, 2.56
>5.3–≤13.4	38	59,012	1.29	0.70, 2.38	1.39	0.75, 2.56
>13.4–≤27.7	32	58,355	1.10	0.59, 2.06	1.24	0.66, 2.32
>27.7	34	57,503	1.19	0.64, 2.21	1.40	0.75, 2.62
				p-trend = 0.72		*p*-trend = 0.71
Supplements						
Folic acid						
No	143	244,880	1.00	Reference	1.00	Reference
Yes	14	15,038	1.60	0.92, 2.77	1.56	0.90, 2.70
Vitamin B_6						
No	133	226,982	1.00	Reference	1.00	Reference
Yes	24	32,936	1.25	0.81, 1.93	1.24	0.80, 1.92
Vitamin B_{12}						
No	140	241,242	1.00	Reference	1.00	Reference
Yes	17	18,676	1.58	0.95, 2.61	1.53	0.93, 2.54
Cigarettes smoked daily						
<14	27	51,792	1.00	Reference	1.00	Reference
14–19	30	42,484	1.36	0.80, 2.28	1.42	0.85, 2.40
20	42	79,806	1.01	0.62, 1.64	1.14	0.70, 1.86
21–25	22	37,950	1.11	0.63, 1.96	1.32	0.75, 2.32
>25	36	47,886	1.45	0.88, 2.38	1.82	1.10, 3.03
				p-trend = 0.30		*p*-trend = 0.05
Years smoked (no.)						
<30	27	65,627	1.00	Reference	1.00	Reference
30–34	16	37,043	1.05	0.57, 1.95	1.13	0.61, 2.10
35–39	31	59,400	1.28	0.76, 2.14	1.20	0.72, 2.02
40–42	37	45,603	1.96	1.21, 3.16	1.49	0.89, 2.50
>42	46	52,125	2.28	1.40, 3.73	1.39	0.75, 2.56
				p-trend = 0.001		*p*-trend = 0.22
Pack-years (no.)						
<22	25	53,176	1.00	Reference	1.00	Reference
22–31	28	52,227	1.14	0.67, 1.96	1.18	0.69, 2.03
32–39	27	48,577	1.19	0.69, 2.04	1.23	0.71, 2.12
40–49	33	52,345	1.35	0.80, 2.27	1.26	0.75, 2.13
>49	44	53,593	1.76	1.08, 2.88	1.66	1.02, 2.72
				p-trend = 0.01		*p*-trend = 0.04
Smoke inhalation						
Never/seldom	14	25,403	1.00	Reference	1.00	Reference
Often	51	99,324	0.86	0.48, 1.55	0.93	0.51, 1.68
Always	92	137,191	1.12	0.64, 1.97	1.25	0.71, 2.20
				p-trend = 0.26		*p*-trend = 0.14
Age started smoking (year of age)						
<17	35	57,260	1.00	Reference	1.00	Reference
17–18	36	62,981	0.93	0.59, 1.48	0.88	0.56, 1.41
19	13	19,914	1.07	0.56, 2.02	0.99	0.52, 1.87
20–21	38	67,786	0.91	0.58, 1.45	0.87	0.55, 1.38
>21	35	51,977	1.10	0.69, 1.75	1.02	0.64, 1.64
				p-trend = 0.67		*p*-trend = 0.85

* Adjusted for age (continuous) and intervention (alpha-tocopherol and beta-carotene supplements).

tile hazards ratio = 0.72; 95 percent confidence interval: 0.41, 1.14; *p*-trend = 0.15) and organ meats (highest compared with lowest quintile hazards ratio = 0.71; 95 percent confidence interval: 0.43, 1.17; *p*-trend = 0.23). Energy-adjusted dietary vitamin B_6, vitamin B_{12}, and methionine (table 2) or alcohol intakes (table 3) were not significantly

associated with pancreatic cancer. There were no significant interactions between alcohol or the smoking variables and energy-adjusted dietary folate, methionine, vitamin B_6, or vitamin B_{12}. Supplemental folic acid, vitamin B_{12}, and vitamin B_6, by contrast, suggested nonsignificant positive associations (table 3) that remained after exclusion of cases from the first 2, 4, and 6 years of follow-up and became statistically significant after 8 years of follow-up. Although the proportion of supplement users was small, supplement users had a lower body mass index; had lower energy and greater alcohol intakes; and more often had a medical history of chronic bronchitis, gallstones, lung emphysema, hypertension, diabetes mellitus, liver cirrhosis, and farmer's lung than did nonsupplement users ($p < 0.05$). However, they did not differ with respect to smoking habits or history of coronary heart disease, pancreatitis, or asthma.

The number of cigarettes smoked daily, after adjusting for age and cumulative smoking dose (pack-years), was significantly positively associated with pancreatic cancer (table 3). The crude smoking duration (years smoked) was also associated with pancreatic cancer. Smoking inhalation showed a weak positive association with disease, and smoking cessation (quit ≥1 year compared with not having quit, age-, and intervention-adjusted hazards ratio = 0.74; 95 percent confidence interval: 0.47, 1.16; data not shown) was nonsignificantly inversely associated with disease.

All hazards ratio estimates were proportional over time and met the assumptions of proportional hazards.

DISCUSSION

This prospective cohort analysis of dietary folate intake (i.e., not from supplements) confirmed the significant protective association between folate and pancreatic cancer observed in the previous nested case-control serologic study (20). By contrast, no protective association was evident for dietary vitamin B_6, vitamin B_{12}, or methionine. Significant positive associations and trends for cigarette smoking were also observed here in the entire cohort. This study's large prospective design with good quality dietary data and a greater number of cases compared with most previous cohort studies is its strength.

Only two observational studies have examined the relation between dietary folate and pancreatic cancer, and both had a case-control design. A population-based study in Adelaide, South Australia, having 104 cases demonstrated a similar significant protective association for greater dietary folate intake: odds ratios of 0.46 and 0.36 for the high tertiles of free and total folic acid with significant trends (27). Silverman et al. (28), in a similar but larger population-based study in the United States with 436 cases, did not observe an association between folate and pancreatic cancer. The latter appeared to use a less detailed food frequency questionnaire (60 food items) than the Australian study (179 food items) or the present study (276 food items) and may have assessed dietary folate intake less precisely, resulting in misclassification and attenuation of risk estimates (27–29). The dietary history questionnaire used in this study has relatively good reliability and validity for measuring dietary nutrients (11). The present findings are also based on prospectively collected data, eliminating temporal ambiguity and recall bias.

Contrary to the protective effect associated with the greater serum vitamin B_6 concentration demonstrated in our nested case-control study (the highest serum tertiles compared with the lowest pyridoxal-5′-phosphate: odds ratio = 0.48; 95 percent confidence interval: 0.26, 0.88; p-trend = 0.02) (20), we observed a nonsignificant association between dietary vitamin B_6 and pancreatic cancer. Approximately half of the control sample in our nested case-control study had low vitamin B_6 status (<30 nmol/liter) (20, 30), despite approximately 95 percent having an energy-adjusted intake of >1.7 mg/day. A number of studies suggest that smokers have lower vitamin B_6 status than do nonsmokers, although the mechanism for this is unclear (14, 16–19). Chemicals inhaled from cigarette smoke may alter vitamin B_6 status independently of dietary intake, for example. None of the smoking variables confounded the association between pyridoxal-5′-phosphate status and pancreatic cancer in our serologic study (20); however, residual confounding is possible. Alternatively, energy-adjusted dietary vitamin B_6 intake may not reflect the absorbed and biologically active dose. Finally, tumors use vitamin B_6 to grow (31), and poorer pyridoxal-5′-phosphate status could be a marker for subclinical disease, particularly as the latency of the cancer is unknown.

Our observed lack of association between dietary methionine or vitamin B_{12} and pancreatic cancer is not surprising as the diet of our population was well above the requirements for protein and vitamin B_{12} to maintain nutritional status (32, 33). Moreover, we did not observe a significant association for alcohol intake and pancreatic cancer. The majority of epidemiologic studies that have examined alcohol consumption have shown no relation with pancreatic cancer (1). Seven of these, however, have been cohort studies with three demonstrating significant positive associations with alcohol (34–40). Because individuals who consume greater quantities of alcohol (alcoholics) often tend to have marginal folate status (13), the inconsistent epidemiologic studies linking alcohol intake to pancreatic cancer may be explained by underlying folate status. In this study, although greater dietary folate was associated with less alcohol intake (table 1), folate did not confound or modify the alcohol association. Consistent with previous studies (3) and despite not having a nonsmoker comparison group, we observed significant positive associations and dose-response trends with cigarette smoking. Cigarette smoke contains chemicals, such as *N*-nitrosamines and aromatic amines, that are likely carcinogens for the pancreas (3).

A number of animal studies support the biologic plausibility of a protective association between folate and pancreatic cancer. The exocrine pancreas has a high, specific requirement for methyl donors (41, 42); therefore, it may be an organ more susceptible to folate deficiency and cancer. Animals treated with an inhibitor of cellular methylation reactions, ethionine, develop acute pancreatitis as a consequence of impaired pancreatic exocrine function (43, 44), and pancreatitis has been associated with pancreatic cancer

(45–47). In addition, animals fed methyl-group-deficient diets (methionine and/or choline with or without folic acid and vitamin B_{12}) have altered pancreatic acinar cell differentiation (42, 48). Finally, folate-deficient animals, compared with those with sufficient folate, have less incorporation of radioactive thymidine into pancreatic DNA (49) and a decreased ratio of *S*-adenosylmethionine (SAM) to *S*-adenosylhomocysteine (SAH) primarily because of elevated SAH (41). The ratio of SAM to SAH is considered to be an important factor in the regulation of methylation reactions.

The variables examined in this study could be correlated to other lifestyle factors not controlled for in our analysis. Those who have higher folate intake and attempt to quit smoking may have healthier lifestyles or be healthier in general. The small proportion of our population who took supplemental folic acid, vitamin B_{12}, and vitamin B_6 tended to have a history of poorer health and may have had less healthy unmeasured behaviors that may account for the suggested greater risks associated with these supplemental vitamins. Alternatively, the timing of folic acid supplementation may be important, particularly in a population at high risk for cancer. In a mouse model for colon cancer, the cancer for which folate has been the most studied, folic acid supplementation given before microscopic neoplastic foci were established protected against intestinal adenoma, while supplementation given after the establishment of neoplastic foci enhanced tumor development (50). Dietary folate intake in our study population, older male smokers, likely represents levels over many years, while supplement intake may represent that taken later in life. The suggested positive association with the supplemental vitamins needs to be interpreted with caution, however, since the number of supplement users was low.

Other limitations to our study include misclassification of exposures and generalizability of our findings to other populations. Measurement error, from nutritional assessment techniques or subjects' changing their diet since baseline, could result in misclassification and biased risk estimates. However, this is less probable at the extremes of intake and in most situations would attenuate risk. Finally, our results may not be generalizable to nonsmoker populations, particularly as smokers have a greater risk of pancreatic cancer and tend to have marginal folate status (15). The majority of our study cohort, as assessed from the study's dietary history questionnaire, consumed less than 400 μg per day (33) and, as estimated from our nested case-control study, had lower concentrations of serum folate compared with other populations (20, 51) and concentrations in the range of deficiency (25 percent with ≤3 nmol/ml). This is likely important to our findings. The protective association between dietary folate and pancreatic cancer needs to be confirmed in other smoking populations and in nonsmokers.

In conclusion, we found statistically significant reductions in risks for exocrine pancreatic cancer associated with greater dietary folate intake in a prospective cohort of older male smokers. A dose-response relation was observed. The magnitude of the risk reduction at the extremes is comparable with that which we observed with serum folate in our previous study (20) and therefore provides additional support for a beneficial role for folate in the prevention of pancreatic cancer. The level of cigarette smoking was also positively associated with cancer risk. Further studies are needed to determine if the observed protective association between dietary folate and pancreatic cancer reflects a cause-and-effect relation.

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